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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>5</sup> :</b> <b>A61K 35/78, C07D 493/04</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 92/11857</b> <b>(43) International Publication Date:</b> 23 July 1992 (23.07.92)
<b>(21) International Application Number:</b> PCT/GB92/00036 <b>(22) International Filing Date:</b> 8 January 1992 (08.01.92)  <b>(30) Priority data:</b> 9100581.9 11 January 1991 (11.01.91) GB  <b>(71) Applicant (for all designated States except US):</b> RHODES TECHNOLOGY LTD. [GB/GB]; 10 Saint Thomas' Place, Cambridgeshire Business Park, Ely, Cambridgeshire CB7 4EX (GB).  <b>(72) Inventor; and</b> <b>(75) Inventor/Applicant (for US only) :</b> RHODES, Alan [GB/GB]; 36 Barton Road, Ely, Cambridgeshire CB7 4HZ (GB).  <b>(74) Agent:</b> BOULT, WADE & TENNANT; 27 Furnival Street, London EC4A 1PQ (GB).		<b>(81) Designated States:</b> AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), MC (European patent), NL (European patent), SE (European patent), US.  <b>Published</b> <i>With international search report.</i> <i>With amended claims.</i>
<b>(54) Title:</b> METHOD FOR THE EXTRACTION OF SESQUITERPENE LACTONES  <b>(57) Abstract</b>  The present invention relates to a method for the extraction of sesquiterpene lactones from the plant <i>Tanacetum parthenium</i> specifically using polar organic solvents and the use of such extracts in pharmaceutical products. It has been found that by utilization of an extraction procedure in accordance with the current invention, a significantly greater amount of the sesquiterpene lactone parthenolide is extracted from <i>Tanacetum parthenium</i> than is the case when using petroleum spirit as the primary extractant, the solvent usually used in previous studies.		

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METHOD FOR THE EXTRACTION OF  
SESQUITERPENE LACTONES

The present invention relates to a method for  
5 the extraction of sesquiterpene lactones from the  
plant Tanacetum parthenium specifically using polar  
organic solvents and the use of such extracts in  
pharmaceutical products.

The plant Tanacetum parthenium (feverfew),  
10 formerly called Chrysanthemum parthenium and also  
known as Midsummer Daisy, Featherfew, Featherfoil,  
Flitwort and Bachelor's Buttons, has a traditional  
reputation for treating a variety of conditions.  
Those claimed most regularly are migraine and  
15 arthritic conditions.

U.K. Patent No 2124486B describes an oil or  
non-polar solvent extraction of a spasmolytically  
active composition which comprises a sesquiterpene  
lactone-containing extract from the plant Tanacetum  
20 parthenium and its use in the treatment of migraine,  
asthmatic, bronchial or arthritic conditions.

Currently, the only preparations of feverfew  
commercially available are tablets or capsules  
containing dried, powdered or comminuted portions of  
25 the plant. These can be obtained through pharmacies  
and healthshops as herbal medicines. However, these  
preparations vary in the part of the plant used and  
in the stated feverfew content.

A crude plant material will of course, contain  
30 many substances which are not of benefit for the  
treatment under consideration and which, in addition  
may have the potential to elicit side effects.  
Moreover, although the dose of crude material can be  
defined, this will probably not be equivalent to a  
35 uniform dose of the active material, whatever this  
active material may be.

Attempts have been made to identify the principle and principally active compound from feverfew. Bohlmann and Zdero (Sesquiterpene Lactones and other constituents from Tanacetum parthenium,  
5 Phytochemistry 1982; 21: 2543-49) reported that following petrol extraction of aerial parts of the plant the major component identified was the sesquiterpene lactone parthenolide. U.K. Patent No. 2124486B identifies parthenolide as the major  
10 component with spasmolytic activity. In 1986 Groenwegen et al (J. Pharm. Pharmacol. 1986; 38:709-712) reported their identification of the constituents of feverfew extracts in relation to their anti-secretory activity, where parthenolide  
15 represents 22% of the active material.

The above work has demonstrated that there is a preponderance of potentially active material found within the leaves of Tanacetum parthenium. In vitro  
pharmacological activity studies suggest that  
20 medicinal activity is shown by the compounds known as sesquiterpene lactones, particularly parthenolide.

It would therefore be of benefit to further investigate the identity of the active constituents of feverfew and to develop a pharmaceutically  
25 acceptable product which retains the medicinal activity of the feverfew plant but in a more refined form than is present in currently commercially available preparations.

Accordingly, the present invention provides a  
30 method for the preparation of a sesquiterpene lactone-containing extract of Tanacetum parthenium which comprises treating Tanacetum parthenium with a polar organic solvent.

The sesquiterpene lactone is derived from the  
35 plant Tanacetum parthenium by a process comprising contacting the plant tissue in a polar organic

solvent for example acetonitrile, methanol, ethanol, isopropanol, ether, ethyl acetate, acetone or a mixture thereof. Ethanol is the preferred solvent, since it is the least toxic with regards to the  
5 residues being left in the final product.

Generally the mixture of plant tissue and polar organic solvent will be left to stand, thereby allowing the extraction to take place. Alternatively, the plant tissue may be exhaustively  
10 extracted with a polar organic solvent in a Soxhlet apparatus or the like. The plant tissue which is extracted in the method of the invention may be fresh, frozen or dried and may be in comminuted form. The extract is then generally separated from  
15 the plant tissue and the solvent removed from the solvent extract by conventional techniques. Following removal of the solvent the remaining primary extract may be further purified by known techniques such as column chromatography and/or  
20 recrystallisation and/or further solvent extraction. The remaining plant tissue may be further extracted using the same or an alternative solvent.

The primary extract may alternatively be utilised as the active material for further  
25 processing into a finished pharmaceutical preparation. For example the material may be mixed with such tableting excipients as are known in the art and processed to obtain tablets. In this way it can be seen that reasonably large doses of feverfew  
30 may be contained within relatively small unit dose preparation. These would have the advantage of being more elegant and palatable than those currently commercially available which contain the dried crude vegetable matter.

35 Surprisingly it has been found that by utilizing an extraction procedure in accordance with

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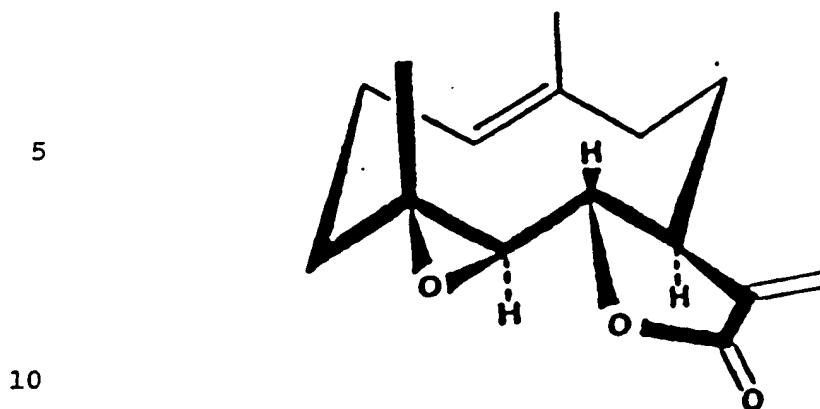
the current invention a significantly greater amount of the sesquiterpene lactone parthenolide is extracted from Tanacetum parthenium than is the case when using petroleum spirit as primary extractant, which appears to have been the solvent usually used in previous studies. In addition, when using ethanol as the solvent, it has been found that camphor, which has previously been identified as a major constituent of petrol extracts of Feverfew, does not appear to be present. This is very surprising in view of the high solubility of camphor in ethanol and is a beneficial feature of the present invention, due to the irritant nature of camphor in pharmaceutical products.

Previous herbal remedies based on the use of feverfew have usually advised the use of the leaves of the plant for medicinal activity. It was therefore also surprising to discover that the parthenolide content of the flowers of the plant tend to be the same or greater than that of the leaves. Conversely, it was also found that the stalk tissue of the plant contains very little parthenolide.

A primary extract of Tanacetum parthenium designed to contain a maximal quantity of sesquiterpene lactones, especially parthenolide, is therefore preferentially prepared using the leaves and flowers of the plant as starting material.

Previous extractions of Tanacetum parthenium have identified many compounds present with anti-secretory and spasmolytically effective activity. The sesquiterpene lactone parthenolide (a germacranolide) appears to be the active ingredient having the greatest abundance in Tanacetum parthenium.

The chemical structure of parthenolide is:



A pharmaceutically active composition comprising a sesquiterpene lactone may be prepared from Tanacetum parthenium and administered solely as a plant extract. However, its pharmaceutical efficacy and popularity are generally increased by the addition of a pharmaceutically acceptable excipient.

In addition to the primary or a further refined extract the final pharmaceutical composition may incorporate other components such as bronchodilators (adrenoceptor stimulants, anti-muscarinic bronchodilators, theophylline or compound bronchodilator preparations), anti-histamines (promethazine, trimeprazine, dimenhydrinate, chlorpheniramine, cyclizine, mequitazine, acrivastine, astemizole, cinnarizine, loratadine or terfenadine) or anti-infective agents (anti-fungal, anti-viral or anti-bacterial agents).

For the treatment of migraine the composition may include, in addition to the sesquiterpene lactone, known anti-migraine preparations (aspirin, paracetamol, ergotamine, dihydroergotamine, metoclopramide, isometheptene mucate, clonidine hydrochloride, methysergide or pizotifen), other analgesics (non-opoid analgesics, opoid analgesics or



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compound analgesic preparations) and/or anti-emetics (hyoscine, anti-histamines, phenothiazines, metoclopramide or clomperidone).

5 A composition containing any of the above-identified compounds may additionally include other anti-arthritic agents: non-steroidal anti-inflammatory drugs, e.g. naproxen, fenbufen, ketoprofen, aspirin and other salicylates; systemic corticosteroids and corticotrophin e.g. prednisolone;  
10 gold, penicillamine, anti-malarials, immuno-suppressants and sulphasalazine.

The sesquiterpene lactone-containing extract in any form may be prepared in the form of a tablet, capsule or liquid suspension for administration  
15 either orally, rectally, parenterally or by inhalation.

In one general embodiment of the present invention, the extraction of 1kg of dried comminuted Feverfew can be accomplished by three extracts of 5,3  
20 and 3 litres of ethanol. The combined extracts can then be distilled in order to concentrate the extract at least twenty times without the parthenolide exceeding its solubility limit.

The invention will now be described in greater  
25 detail by way of the following specific examples.

#### EXAMPLE 1

##### Ether Extraction

30

Feverfew (490g) was extracted with 2 litres of ether in a Winchester bottle which was maintained at room temperature for 5 days. The ether was poured out, filtered and evaporated in vacuo, whilst  
35 maintaining the temperature below 40°C. The residue was partially purified by column

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chromatography to give parthenolide (2.7g), impure by thin layer chromatography. Further chromatography, eluting with 40-60°C petrol - ethyl acetate as a 3:2 mixture, gave 1.45g, essentially one spot on the

5 thin layer chromatogram. This material (1.45g) was dissolved in ether (approximately 25ml), filtered and the volume reduced to 10ml. After the solution had been chilled overnight the crystals that had separated were filtered off and washed with cold

10 ether (approximately 10ml). Yield of parthenolide 0.74g (slight green tint), m.p. 112-113°C (lit. 116.5-117°C). This sample had the same Rf on a silica gel chromatogram as parthenolide using ether or 40-60°C petrol - ethyl acetate in a 3:2 mixture.

15 Removal of the solvent from the filtrate left a residue of 0.60g.

#### EXAMPLE 2

##### 20 Acetonitrile Extraction

Feverfew (496g) was extracted with 2 litres of acetonitrile as described in Example 1. Removal of the solvent left a residue of 30.0g, which was

25 chromatographed with 40-60°C petrol - ethyl acetate in a 3:2 mixture, as eluent. The product (1.7g) was crystallised from ether (15ml) to give slightly coloured parthenolide (1.02g). Recrystallisation from ether (15ml) produced colourless crystals

30 (0.72g), m.p. 113-114°C.

#### EXAMPLE 3

The quantity of parthenolide extracted from

35 samples of dried comminuted feverfew leaves using various solvents was determined using a sensitive

high pressure liquid chromatographic analytical procedure.

The results are given in Table 1 below:

5

TABLE 1

Amount (mg) of parthenolide extracted from 100mg portions of dried comminuted feverfew leaves

10

	Solvent	1st Extract (5ml)	2nd Extract (5ml)	Total
15	Petroleum spirit extract (non-polar organic solvent for comparison)	0.22	0.12	0.34
20	Water (Polar inorganic solvent for comparison)	0.24	0.15	0.39
25	Isopropyl alcohol (Polar organic solvent)	0.59	0.29	0.88
30	Acetonitrile (Polar organic solvent)	0.66	0.17	0.83

EXAMPLE 4

35

The parthenolide contents of methanol extracts of 1g

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portions of various feverfew tissues were assessed using a sensitive high pressure liquid chromatographic analytical procedure. The results are given in Table 2 below.

5

TABLE 2

Amount (mg) of parthenolide extracted into methanol from 1g portions of various feverfew tissues

10

Tissue	1st Extract (100ml)	2nd Extract (100ml)	Total (mg)
Leaves	4.3	0.2	4.5
Flowers	14.6	0.6	15.2
Stalks	0.2	-	0.2

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CLAIMS:

1. A method for the preparation of a  
sesquiterpene lactone-containing extract of Tanacetum  
5 parthenium which comprises treating Tanacetum  
parthenium with a polar organic solvent.
2. A method as claimed in claim 1 wherein the  
polar organic solvent is acetonitrile, methanol,  
10 isopropanol, ether, ethyl acetate, acetone, or  
mixtures thereof.
3. A method as claimed in claim 1 wherein the  
polar organic solvent is ethanol.  
15
4. A method as claimed in any preceeding claim  
wherein the sesquiterpene lactone is purified by  
differential extraction.
- 20 5. A method as claimed in any one of claims 1 to  
4 wherein the Tanacetum parthenium tissue treated is  
flower, leaf or a combination thereof.
6. A method as claimed in any preceding claim  
25 wherein the sesquiterpene lactone extracted is  
parthenolide.
7. A sesquiterpene lactone-containing extract of  
Tanacetum parthenium which has been prepared by a  
30 method as claimed in any one of the preceding claims.
8. A pharmaceutically active composition  
comprising a sesquiterpene lactone-containing extract  
from the plant Tanacetum parthenium as claimed in  
35 claim 7.

9. A composition as claimed in claim 8 which additionally contains a pharmaceutically acceptable excipient.

5 10. A composition as claimed in claim 8 or claim which additionally contains at least one known bronchodilator, anti-histamine or anti-infective agent

10 11. A composition as claimed in any one of claims 8 to 10 which additionally contains at least one known anti-migraine ingredient, analgesic or anti-emetic.

15 12. A composition as claimed in any one of claims 8 to 11 which additionally contains at least one anti-arthritic agent.

20 13. A composition as claimed in any one of claims 8 to 12 which is in the form of a tablet, capsule or liquid solution or suspension.

25 14. A composition as claimed in any one of claims 8 to 12 which is suitable for administration orally, rectally, parenterally or by inhalation.

30 15. A method for the production of a sesquiterpene lactone - containing extract of Tanacetum parthenium substantially as hereinbefore described with reference to any one of the Examples.

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## AMENDED CLAIMS

[received by the International Bureau on 7 July 1992 (07.07.92);  
original claims 1-15 replaced by amended claims 1-12 (2 pages)]

1. A method for the preparation of a  
sesquiterpene lactone-containing extract of Tanacetum  
5 parthenium which comprises treating Tanacetum  
parthenium with the solvent methanol, ethanol or  
isopropanol.
2. A method as claimed in claim 1 wherein the  
10 sesquiterpene lactone is purified by differential  
extraction.
3. A method as claimed in claim 1 or claim 2  
wherein the Tanacetum parthenium tissue treated is  
15 flower, leaf or a combination thereof.
4. A method as claimed in any preceding claim  
wherein the sesquiterpene lactone extracted is  
parthenolide.  
20
5. A sesquiterpene lactone-containing extract of  
Tanacetum parthenium which has been prepared by a  
method as claimed in any one of the preceding claims.
- 25 6. A pharmaceutically active composition  
comprising a sesquiterpene lactone-containing extract  
from the plant Tanacetum parthenium as claimed in  
claim 5.
- 30 7. A composition as claimed in claim 6 which  
additionally contains a pharmaceutically acceptable  
excipient.
8. A composition as claimed in claim 6 or claim 7  
35 which additionally contains at least one known  
bronchodilator, anti-histamine or anti-infective agent.

9. A composition as claimed in any one of claims 6 to 8 which additionally contains at least one known anti-migraine ingredient, analgesic or anti-emetic.

5 10. A composition as claimed in any one of claims 6 to 9 which additionally contains at least one anti-arthritic agent.

10 11. A composition as claimed in any one of claims 6 to 10 which is in the form of a tablet, capsule or liquid solution or suspension.

15 12. A composition as claimed in any one of claims 6 to 10 which is suitable for administration orally, rectally, parenterally or by inhalation.

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## INTERNATIONAL SEARCH REPORT

PCT/GB 92/00036

International Application No

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)<sup>6</sup>

According to International Patent Classification (IPC) or to both National Classification and IPC  
 Int.Cl. 5 A61K35/78; C07D493/04

## II. FIELDS SEARCHED

Minimum Documentation Searched<sup>7</sup>

Classification System

Classification Symbols

Int.Cl. 5

A61K ; C07D

Documentation Searched other than Minimum Documentation  
 to the Extent that such Documents are Included in the Fields Searched<sup>8</sup>

III. DOCUMENTS CONSIDERED TO BE RELEVANT<sup>9</sup>

Category <sup>10</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
Y	CHEMICAL ABSTRACTS, vol. 114, no. 6, 11 February 1991, Columbus, Ohio, US; abstract no. 49417C, BANTHORPE, D. ET AL.: 'Parthenolide and other volatiles in the flowerheads of Tanacetum parthenium(L.) Schultz Bip.' page 412 ; see abstract & FLAVOUR FLAGRANCE J. vol. 5, no. 3, 1990, ENG pages 183 - 185;	1,8-14
Y	EP,A,0 098 041 (JOHNSON) 11 January 1984 see page 6. - page 26; claims & GB,A,2 124 486 22 February 1984 cited in the application --- -/-	1,8-14

\* Special categories of cited documents : <sup>10</sup>

\* "A" document defining the general state of the art which is not considered to be of particular relevance

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## IV. CERTIFICATION

Date of the Actual Completion of the International Search

14 APRIL 1992

Date of Mailing of this International Search Report

06.05.92

International Searching Authority

EUROPEAN PATENT OFFICE

Signature of Authorized Officer

DELANGHE L. L. M.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		Relevant to Claim No.
Category <sup>a</sup>	Citation of Document, with indication, where appropriate, of the relevant passages	
P,X	CHEMICAL ABSTRACTS, vol. 115, no. 15, 14 October 1991, Columbus, Ohio, US; abstract no. 155015 A, HAUSEN, BJOERN M.: 'A simple method of isolating parthenolide from Tanacetum and other sensitizing plants' page 534 ; see abstract	1-3,5-7, 15
P,Y	& CONTACT DERMATITIS vol. 24, no. 2, 1991, ENG pages 153 - 155; ---	9-14

# ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

GB 9200036  
SA 55163

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.  
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		AU-A- 1464683	24-11-83
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		US-A- 4758433	19-07-88
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		JP-A- 59001425	06-01-84
		US-A- 4758433	19-07-88

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